



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :

G01N 33/543, 21/00

A1

(11) International Publication Number:

WO 98/10288

(43) International Publication Date:

12 March 1998 (12.03.98)

(21) International Application Number: PCT/GB97/02347

(22) International Filing Date: 2 September 1997 (02.09.97)

(30) Priority Data:

9618635.8

6 September 1996 (06.09.96) GB

(71) Applicant (for all designated States except US): THERMO  
FAST U.K. LIMITED [GB/GB]; Saxon Way, Bar Hill,  
Cambridge CB3 8SL (GB).

(72) Inventors; and

(75) Inventors/Applicants (for US only): MOLLOY, James, Oscar  
[GB/GB]; 85 High Street, Cheveley, Newmarket, Suffolk  
CB8 9DQ (GB). MAULE, Colin, Hugh [GB/GB]; 76  
Richmond Road, Cambridge CB4 3PT (GB).(74) Agent: JONES, Stephen, Anthony; Lewis & Taylor, 144 New  
Walk, Leicester LE1 7JA (GB).(81) Designated States: JP, US, European patent (AT, BE, CH, DE,  
DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

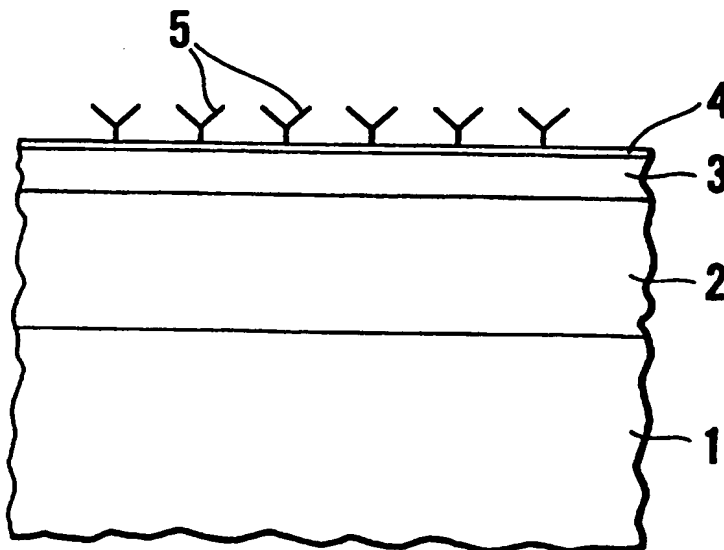
## Published

*With international search report.**Before the expiration of the time limit for amending the  
claims and to be republished in the event of the receipt of  
amendments.*

(54) Title: IMPROVEMENTS IN OR RELATING TO SENSORS

## (57) Abstract

A sensor device (1-5) has a sensing surface on which, in use, first molecules (5) are immobilized. The first molecules (5) are capable of interaction with second molecules which may be present in a sample of fluid applied to the sensing surface, such interaction resulting in a measurable change of some physical property of the sensor device. The sensing surface is coated with a layer (4) of diamond-like carbon to protect and preserve the integrity of the sensing surface.



**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

Title - Improvements in or relating to sensors

This invention relates to improvements in or relating to sensors, and in particular to those sensors termed biosensors, ie devices for the analysis and investigation of biological or biologically active species such as antigens and antibodies, enzymes, substrates, proteins, haptens, whole cells and cellular fragments and nucleic acids.

Many devices for the automatic determination of biochemical analytes in solution have been proposed in recent years. Typically, such devices (biosensors) include a sensitised coating layer which is located in the evanescent region of a resonant field. Typically, the coating layer comprises a layer of biological molecules chemically linked to the surface, either directly or via an intermediate linking molecule, or immobilised within a matrix of, for instance, hydrogel molecules bound to the surface.

Detection of the molecule under investigation ("the analyte") typically utilizes optical techniques such as, for example, surface plasmon resonance (SPR) or frustrated total reflection (FTR), and is based on changes in the thickness and/or refractive index of the coating layer resulting from interaction of that layer with the analyte. This causes a change in the properties of the sensor, eg a change in the angular position of the resonance. Other forms of biosensor include devices with semiconducting surfaces, the electrical properties of the device being monitored and, notably, acoustic devices in which changes in surface bulk loading are detected.

Since the measurements made using biosensors of the types described are essentially measurements of events or changes occurring at the sensitised surface of the device, it is critical to the accuracy and reliability of the measurements that the integrity of that surface is maintained. In an SPR or FTR sensor, for example, the device monitors the resonating properties of a structure the natural frequency of which is altered as changes take place at its surface. If the sample can alter the bulk of the structure in a non-specific manner then the integrity of the measurement is destroyed. In practice, maintenance of surface integrity may not be achieved and this gives rise to errors in the experimental results and/or greatly limits the useful life of the sensor devices. For example, repeated application of reagents to the surface (as is inevitable in

a series of measurements) may result in attrition of the surface, with a consequent unpredictable change in properties. The surface may be somewhat porous, with the result that reagents may be absorbed, again changing the properties of the device. Chemical linkages between the surface and the molecules immobilized on it may also become broken in the course of chemical treatment.

There has now been devised an improvement to sensors of the kind generally described above which overcomes or substantially mitigates the disadvantages of the prior art.

According to the invention, a sensor device has a sensing surface on which, in use, first molecules are immobilized, the first molecules being capable of interaction with second molecules which may be present in a sample of fluid applied to the sensing surface, such interaction resulting in a measurable change of some physical property of the sensor device, wherein the sensing surface is coated with a layer of diamond-like carbon.

The sensor device according to the invention is advantageous primarily in that the layer of diamond-like carbon (DLC) protects and preserves the integrity of the sensing surface. The device is impervious to the reagents and fluids with which it is, in use, contacted. Problems of attrition of the surface and porosity are reduced, and linkages of the first molecules to the surface are more stable. Furthermore, and particularly importantly, by appropriate control of the composition of the diamond-like carbon layer (as described below) a wide variety of functionalities may be incorporated into it in a thickness-dependent manner.

DLC is a dense, partially  $sp^3$  bonded form of amorphous carbon. Its atomic structure consists of a network of  $sp^3$  and  $sp^2$  sites, the connectivity of the  $sp^3$  sites controlling the mechanical properties of the material. DLC is conventionally used as a hard coating material, ie to confer "diamond-like" properties such as mechanical hardness and low friction on substrate materials. Since the purpose of the DLC layer used in the present invention is not primarily to confer a high degree of hardness on the active surface of the sensor device, the layer may have a hardness which is considerably less than that achieved in conventional applications of DLC.

The DLC layer may be formed by plasma deposition or chemical vapour deposition techniques. Typically, monomeric starting materials in the gas phase are introduced into a vacuum chamber containing a pair of electrodes. The device to be coated is supported in the chamber on one of the electrodes and a radiofrequency or microwave discharge is applied.

Generally, the starting material includes a hydrocarbon, most preferably methane. However, in principle any suitable hydrocarbon may be used, eg ethylene, acetylene, ethane, or aromatic species such as toluene and styrene. Mixtures of starting materials may be used to give desired physical properties.

It may also be desirable to incorporate other chemical functionality in the DLC layer. For instance, by introducing  $\text{CH}_3\text{NH}_2$  gas in the final stages of the deposition, a DLC layer may be formed with a surface which includes amino groups. Such groups may be useful for the direct immobilization of biomolecules. Similarly, inclusion of carboxylate-containing species in the vapour may give rise to a surface with carboxylate functionality. The starting materials may also include small quantities of gases such as argon, neon, nitrogen, oxygen or helium. Appropriate combinations of starting materials may also be used to produce DLC layers having particularly hydrophobic or hydrophilic properties.

Because the polymerisation reaction is essentially simple, a high degree of control can be exercised over the chemical and physical nature of the DLC layer, enabling the properties of that layer to be easily tailored to the particular application for which the sensor device is intended. One physical parameter which is important is the density of the DLC layer, which is determined largely by the proportion of  $\text{sp}^3$  to  $\text{sp}^2$  hybridized carbon. For optical sensors, a dense DLC layer is desirable to minimise the thickness of the DLC layer necessary to provide the necessary degree of protection without adversely affecting the optical properties of the sensor. The density (and thickness) of the DLC layer may be less important, or not at all important, for non-optical sensors.

The DLC layer should have a thickness which is sufficient to confer the desired degree of protection on the sensing surface of the sensor device. The thickness of the DLC layer can be

controlled by appropriate choice of the operating parameters of the deposition apparatus, notably the period for which the deposition is carried out. In general, the thickness should be no greater than the minimum required, so as to avoid any possible deleterious effects of the DLC layer on the properties, eg the sensitivity, of the sensor device. Typically, the DLC layer will have a thickness of less than 100nm, more preferably less than 50nm, and particularly less than 20nm. A thickness of greater than 1nm, and generally greater than 5nm will normally be required. The thickness is most preferably of the order of 10nm.

The DLC layer may be applied directly to the surface of the sensor device which it is desired to protect. However, since the DLC layer may not adhere sufficiently well to the material of that surface, it may be necessary to apply first a thin layer of another material to which the DLC layer will adhere well.

The sensor device according to the invention may be of any type, eg an optical sensor or any other form of sensor in which changes at the sensing surface result in a measurable change of physical property. One preferred form of optical sensor is based on frustrated total reflection. The principles of frustrated total reflection (FTR) are well known; the technique is described, for example, by Bosacchi and Oehrle [Applied Optics (1982), 21, 2167-2173]. An FTR device for use in immunoassay is disclosed in European Patent Application No 0205236A and comprises a cavity layer bounded on one side by the sample under investigation and on the other side by a spacer layer which in turn is mounted on a substrate. The substrate-spacer layer interface is irradiated with monochromatic radiation such that total reflection occurs, the associated evanescent field penetrating through the spacer layer. If the thickness of the spacer layer is correct and the incident parallel wave vector matches one of the resonant mode propagation constants, the total reflection is frustrated and radiation is coupled into the cavity layer. The cavity layer must be composed of material which has a higher refractive index than the spacer layer and which is transparent at the wavelength of the incident radiation.

An FTR sensor will generally include an optical structure comprising

- a) a cavity layer of transparent dielectric material of refractive index  $n_3$ ,
- b) a dielectric substrate of refractive index  $n_1$ , and

c) interposed between the cavity layer and the substrate, a dielectric spacer layer of refractive index  $n_2$ .

In use, the interface between the substrate and the spacer layer is irradiated with light such that internal reflection occurs. Resonant propagation of a guided mode in the cavity layer will occur, for a given wavelength, at a particular angle of incidence of the exciting radiation.

The angular position of the resonant effect depends on various parameters of the sensor device, such as the refractive indices and thicknesses of the various layers. It is a pre-requisite that the refractive index  $n_3$  of the cavity layer and the refractive index  $n_1$  of the substrate should both exceed the refractive index  $n_2$  of the spacer layer. Also, since at least one mode must exist in the cavity to achieve resonance, the cavity layer must exceed a certain minimum thickness.

The cavity layer is preferably a thin-film of dielectric material. Suitable materials for the cavity layer include silicon nitride, hafnium dioxide, zirconium dioxide, titanium dioxide, aluminium oxide and tantalum oxide.

The dielectric spacer layer must have a lower refractive index than both the cavity layer and the substrate. The layer may, for example, comprise an evaporated or sputtered layer of magnesium fluoride. In this case an infra-red light injection laser may be used as light source. The light from such a source typically has a wavelength around 600-800nm. Other suitable materials include lithium fluoride and silicon dioxide.

The refractive index of the substrate ( $n_1$ ) must be greater than that ( $n_2$ ) of the spacer layer but the thickness of the substrate is generally not critical.

By contrast, the thickness of the cavity layer must be so chosen that resonance occurs within an appropriate range of coupling angles. The spacer layer will typically have a thickness of the order of several hundred nanometres, say from about 200nm to 2000nm, more preferably 500 to 1500nm, eg 1000nm. The cavity layer typically has a thickness of a few tens of nanometres, say 10 to 200nm, more preferably 30 to 150nm, eg 100nm.

It is particularly preferred that the cavity layer has a thickness of 30 to 150nm and comprises a material selected from silicon nitride, hafnium dioxide, zirconium dioxide, titanium dioxide, tantalum oxide and aluminium oxide, and the spacer layer has a thickness of 500 to 1500nm and comprises a material selected from magnesium fluoride, lithium fluoride and silicon dioxide, the choice of materials being such that the refractive index of the spacer layer is less than that of the cavity layer.

Preferred materials for the cavity layer and the spacer layer are silicon nitride and silicon dioxide respectively.

At resonance, the incident light is coupled into the cavity layer by FTR, propagates a certain distance along the cavity layer, and couples back out (also by FTR). The propagation distance depends on the various device parameters but is typically of the order of 1 or 2mm.

At resonance the reflected light will undergo a phase change, and it is this which may be detected. Alternatively, as described in International Patent Application No WO 92/03720 the cavity layer and/or spacer layer may absorb at resonance, resulting in a reduction in the intensity of the reflected light.

The DLC layer is preferably formed on the surface of the cavity layer after the spacer layer and cavity layer have been applied to the substrate. In addition to the DLC layer, the cavity layer or the cavity layer and the spacer layer may be formed by plasma deposition or chemical vapour deposition techniques. The substrate may, for example, be placed in the deposition chamber and the spacer layer, cavity layer and DLC layer formed sequentially.

Another particular form of sensor which may be mentioned is the type of optical sensor disclosed in copending International Patent Application WO 97/29362. Such a device comprises a substrate having a waveguide formed on at least part of the surface thereof, the waveguide having a first major surface which constitutes an interface between the waveguide and the substrate and a second major surface upon which the first molecules are immobilized, at least a region of the first and/or second major surface being formed with a periodic refractive index



modulation. When such a device is modified in accordance with the present invention, the DLC layer is applied to the second major surface of the waveguide, ie between the waveguide and the first molecules.

With such a device, as described in WO 97/29362, high intensity of reflected light may be observed. Such high reflection may be termed "anomalous" or "abnormal" reflection. Interaction of the molecular species immobilized on the waveguide surface with analyte molecules in a sample which is contacted with the waveguide causes a local change in refractive index in the vicinity of the waveguide surface. This in turn changes the angle of incidence or wavelength at which the reflection maximum occurs, providing a sensitive indicator of the chemical interaction taking place at the surface.

The periodic refractive index modulation is preferably a surface relief profile or a grating formed in the surface of the substrate to which the waveguide coating is applied and/or in the surface of the waveguide on which the first molecules are immobilized. The periodic refractive index modulation may be formed in one or both major surfaces of the waveguide.

The grating may have a variety of forms. For example, it may be sinusoidal, rectangular, triangular (saw-tooth) or trapezoidal.

The substrate is conveniently a chip, eg of glass or silica, and, in use, the superstrate is most commonly an aqueous sample. The waveguide is preferably of relatively high refractive index, e.g. a material having a refractive index of, say, 1.80 to 2.50. Suitable materials for the waveguide include hafnium dioxide, silicon nitride, tantalum pentoxide and titanium oxide.

The optimal physical dimensions of the sensor device, grating etc will depend on the wavelength of the incident light. In the following description, the values given for the waveguide thickness, grating depth and period, light beam diameter etc encompass those suitable for commonly-used wavelengths, eg a wavelength of 633nm.

Typically, the waveguide may have a thickness of the order of 50nm to 300nm, more preferably

100nm to 200nm. We particularly prefer the thickness of the waveguide to be in the range 140nm to 180nm.

The depth of the periodic refractive index modulations (e.g. the corrugations in the surface of the substrate) is preferably less than 50nm, more preferably less than 25nm, eg typically 2nm to 20nm or 5nm to 10nm. The period of the grating is typically 300nm to 1000nm, more preferably 600nm to 1000nm.

For use in the analysis of biochemical species, the first molecules immobilized, in use, on the sensing surface of the sensor device (ie on the DLC layer) will generally be biomolecules, eg specific binding partners for the second molecules (the analyte). The first molecules may be bound to the surface by methods which are well known to those skilled in the art. The first molecules may be covalently bound to the DLC layer, either directly or via linking molecules, or may be bound to a matrix, eg a porous matrix of a hydrogel such as agarose or dextran, which is itself bound to the DLC layer. Examples of pairs of classes of molecules, one of which may be immobilized as first molecule for interaction with the other as second molecule are:

antigen / antibody

hormone / hormone receptor

polynucleotide strand / complementary strand

avidin / biotin

enzyme / substrate

carbohydrate / lectin

The sensor device may be used for the quantitative or qualitative determination of the second molecule in a sample applied to the sensing surface, or may be used to study the interaction of the second molecules with the immobilized first molecules.

The invention will now be described in greater detail, by way of illustration only, with reference to the accompanying drawings, in which

Figure 1 is a schematic cross-sectional view of a first sensor device according to the invention;

Figure 2 is a schematic view of chemical vapour deposition apparatus used in the manufacture of the sensor device of Figure 1; and

Figure 3 is a schematic cross-sectional view of a second sensor device according to the invention.

Referring first to Figure 1, a biosensor based on the principle of frustrated total reflection (FTR) comprises a substrate 1 in the form of a glass chip, on the surface of which are formed successively a spacer layer 2 of silicon oxide and a cavity layer 3 of silicon nitride. The spacer layer 2 has a thickness of approximately 700nm and the cavity layer 3 a thickness of approximately 100nm.

The surface of the cavity layer 3 is coated with a protective layer 4 of diamond-like carbon of approximate thickness 10nm. Antibodies 5 are covalently bound to the surface of the protective layer 4.

Figure 2 shows apparatus used for the deposition of the spacer layer 2, cavity layer 3 and protective layer 4 on the substrate 1. The apparatus comprises a vacuum chamber 10 with an exhaust port 11 formed in its base, the exhaust port 11 being connected to a pump 12. Gases are fed to the chamber 10 through an inlet conduit 13, via a mass flow controller 14. The inlet conduit 13 terminates in a "shower-head" arrangement 15 which constitutes a first electrode.

A support 16 is positioned below the shower-head 15 and constitutes a second electrode. The support is connected to a 13.65 MHz radiofrequency generator 18, via a matching unit 19.

In use, the substrate 1 is positioned on the support 16 and the sequence of layers 2,3,4 built up sequentially by chemical vapour deposition. A gas is passed through the shower-head 15 into the space between the shower-head 15 and the support 16. A plasma is formed in that space and deposition of ions created in the plasma takes place. First, the spacer layer 2 is formed by introduction of an appropriate precursor gas through the shower-head 15 and appropriate setting

of operating parameters. The precursor gas and operating parameters are then changed to form the cavity layer 3. A further change of gas and operating parameters leads to formation of the protective layer 4, as described below.

In order to form the protective layer 4, methane gas is fed through the inlet conduit 13 as indicated by the arrow. The operating parameters which are used to control the extent and rate of deposition are principally the operating temperature, the flow rate of gas into the chamber 10, the pressure within the chamber 10 and the applied bias voltage. A typical set of parameters is:

Temperature	room temperature
Flow rate	10 sccm (standard cubic centimetres / minute)
Pressure	50 mTorr
Self-generated Bias voltage	70 V

With these operating conditions, deposition of a protective layer 4 having a thickness of approximately 10nm typically takes about 5 minutes.

The arrangement described above, in which the workpiece (the substrate 1) is placed on the driven electrode is unusual; a more conventional deposition arrangement being one in which the other electrode is driven.

Finally, Figure 3 shows a second form of sensor device according to the invention. This device is of the type described in WO 97/29362 and comprises a substrate in the form of a chip 21 (eg of glass or silica) approximately 7mm square and 2mm in thickness. The chip 21 has a refractive index of 1.46. Coated on the upper surface of the chip 21 is a waveguide 22.

The interface between the chip 21 and the waveguide 22 is formed with a periodic relief profile or grating 23 (the grating 23 is shown as being sinusoidal though in practice it may be generally rectangular). The waveguide 22 is formed by deposition on the chip 21 and a corresponding relief profile 24 may thus be formed also on the upper surface of the waveguide 22. The upper surface of the waveguide 22 is coated with a protective layer 26 of diamond-like carbon of

approximate thickness 10nm (by a process similar to that described above in relation to the embodiment of Figure 1). A layer 25 of biomolecules, eg antibodies, is immobilized on the protective layer 26 in a known manner.

Claims

1. A sensor device with a sensing surface on which, in use, first molecules are immobilized, the first molecules being capable of interaction with second molecules which may be present in a sample of fluid applied to the sensing surface, such interaction resulting in a measurable change of some physical property of the sensor device, wherein the sensing surface is coated with a layer of diamond-like carbon (DLC).
2. A sensor device according to claim 1, wherein the DLC layer is formed by a plasma deposition or chemical vapour deposition technique.
3. A sensor device according to claim 2, wherein in said technique monomeric starting materials in the gas phase are introduced into a vacuum chamber containing a pair of electrodes, a device to be coated being supported in the chamber on one of the electrodes, and a radiofrequency or microwave discharge is applied.
4. A sensor device as claimed in claim 3, wherein the starting material includes a hydrocarbon, most preferably methane.
5. A sensor device as claimed in any preceding claim, wherein the DLC layer has a surface which includes amino groups.
6. A sensor device as claimed in any preceding claim, wherein the DLC layer has a surface which includes carboxylate groups.
7. A sensor device as claimed in any preceding claim, wherein the DLC layer has a thickness of less than 100nm.
8. A sensor device as claimed in any preceding claim, wherein the DLC layer has a thickness of greater than 1nm.

9. A sensor device as claimed in any preceding claim, wherein the DLC layer has a thickness of the order of 10nm.
10. A sensor device as claimed in any preceding claim, which is an optical sensor based on frustrated total reflection, and comprising
- a) a cavity layer of transparent dielectric material of refractive index  $n_3$ ,
  - b) a dielectric substrate of refractive index  $n_1$ , and
  - c) interposed between the cavity layer and the substrate, a dielectric spacer layer of refractive index  $n_2$ .
11. A sensor device as claimed in any one of claims 1 to 9, which is an optical sensor comprising a substrate having a waveguide formed on at least part of the surface thereof, the waveguide having a first major surface which constitutes an interface between the waveguide and the substrate and a second major surface upon which the DLC layer is applied, at least a region of the first and/or second major surface being formed with a periodic refractive index modulation.
12. A method for the analysis of biochemical species in a fluid, which method comprises contacting a sample of the fluid with molecules immobilized on the DLC layer of a sensor as claimed in any one of claims 1 to 11.
13. A method of forming a sensor as claimed in any one of claims 1 to 11, which method comprises supporting a sensor device on one of a pair of electrodes within a vacuum chamber, introducing monomeric starting material in the gas phase into the vacuum chamber and applying a radiofrequency or microwave discharge between the electrodes.

1/2

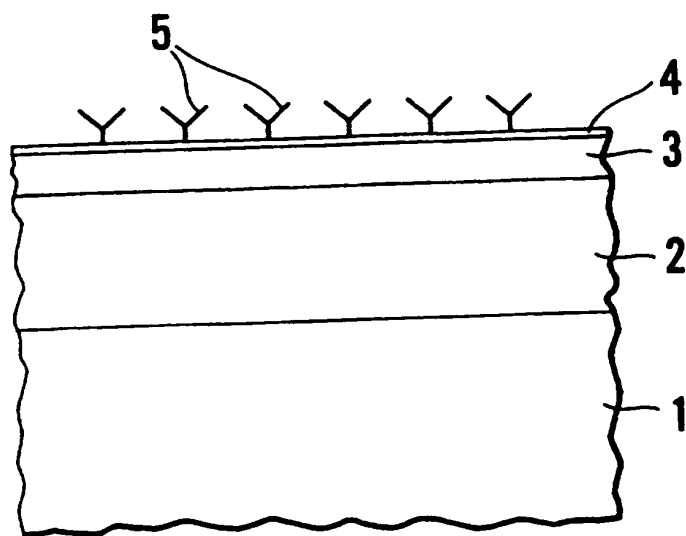


Fig. 1

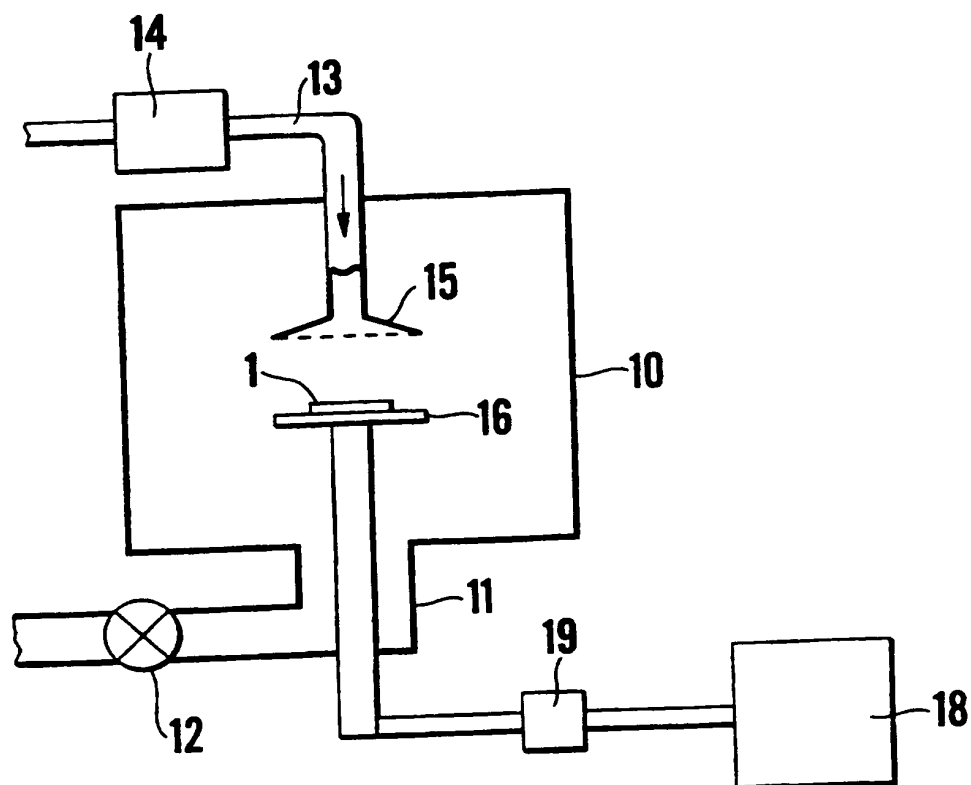


Fig. 2

SUBSTITUTE SHEET (RULE 26)



2/2

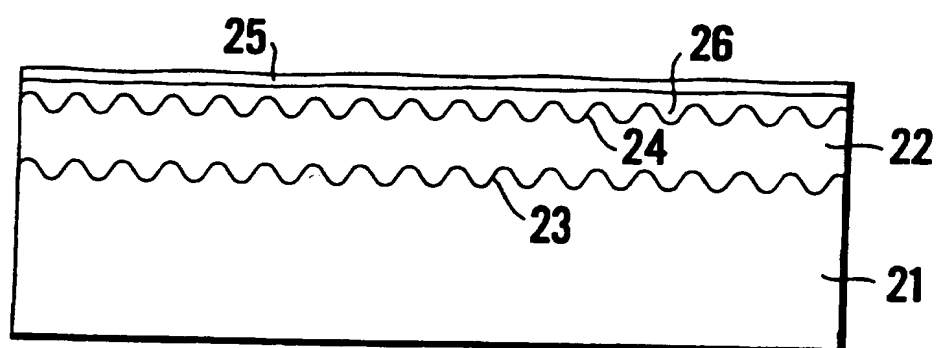


Fig.3

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/GB 97/02347

**A. CLASSIFICATION OF SUBJECT MATTER**  
IPC 6 G01N33/543 G01N21/00

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 93 24828 A (UNIV MANCHESTER ; VADGAMA PANKAJ MADGANLAL (GB); HIGSON SEAMUS PATR) 9 December 1993 see the whole document ---	1-13
Y	DATABASE WPI Section Ch, Week 9432 Derwent Publications Ltd., London, GB; Class L03, AN 94-260358 XP002049537 & JP 06 191 992 A (IDEMITSU PETROCHEM CO) , 12 July 1994 see abstract --- -/--	1,2

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

\* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

15 December 1997

Date of mailing of the international search report

14/01/1998

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

Moreno, C

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 97/02347

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	S. P. J. HIGSON & P. M. VADGAMA: "Diamond like carbon films for enzyme electrodes; characterisation of novel overlaying permselective barriers." ANALYTICA CHIMICA ACTA, vol. 300, no. 1-3, 1995, pages 85-90, XP002049535 see the whole document	1,2
Y	S. P. J. HIGSON & P. M. VADGAMA: "Diamond-like carbon coated microporous polycarbonate as a composite barrier for a glucose enzyme electrode." ANALYTICA CHIMICA ACTA, vol. 271, no. 1, 8 January 1993, pages 125-133, XP002049536 see the whole document	1,2
A	J. FRANKS: "Preparation and properties of diamond-like carbon films" JOURNAL OF VACUUM SCIENCE & TECHNOLOGY, vol. 7, no. 3, May 1989, pages 2307-2310, XP002050186 see the whole document	1
A	US 5 552 272 A (BOGART GREGORY R) 3 September 1996 see the whole document	1
A	EP 0 205 236 A (PLESSEY OVERSEAS) 17 December 1986 cited in the application see the whole document	1
A	WO 92 03720 A (FISONS PLC) 5 March 1992 cited in the application see the whole document	1
P,A	WO 97 29362 A (THERMO FAST UK LIMITED ;LYNDIN NIKOLAI MIKHAILOVICH (RU); SYCHUGOV) 14 August 1997 cited in the application see the whole document	1

# INTERNATIONAL SEARCH REPORT

information on patent family members

International Application No

PCT/GB 97/02347

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9324828 A	09-12-93	AU 676678 B	20-03-97
		AU 4079693 A	30-12-93
		CA 2136856 A	09-12-93
		EP 0647318 A	12-04-95
		JP 8501144 T	06-02-96
		NZ 252235 A	26-07-96
		US 5531878 A	02-07-96
US 5552272 A	03-09-96	NONE	
EP 0205236 A	17-12-86	DE 3687543 A	04-03-93
		GB 2174802 A, B	12-11-86
		JP 2110874 C	21-11-96
		JP 7117486 B	18-12-95
		JP 61292044 A	22-12-86
		US 4857273 A	15-08-89
WO 9203720 A	05-03-92	DE 69115493 D	25-01-96
		DE 69115493 T	04-07-96
		EP 0543831 A	02-06-93
		JP 5509402 T	22-12-93
		US 5434663 A	18-07-95
WO 9729362 A	14-08-97	NONE	